

• 综 述 •

## 轮状病毒 VP4 亚单位疫苗研究进展

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**摘 要:** 轮状病毒是全球范围内导致 5 岁以下婴幼儿严重腹泻的主要病原体, 造成了巨大的经济负担和社会负担。疫苗预防接种是控制轮状病毒感染最为有效的手段, 但在轮状病毒导致的死亡率较高的非洲和亚洲部分低收入国家, 目前已经上市的轮状病毒疫苗的有效性较低, 且会增加肠套叠的风险。更加安全、有效的轮状病毒疫苗对于降低轮状病毒感染导致的发病率和死亡率具有重要意义。目前, 各国科研人员试图从多个方面提高轮状病毒疫苗的有效性, 非复制型基因工程亚单位疫苗是目前轮状病毒疫苗研究的主要方向。文中就目前轮状病毒亚单位疫苗, 特别是基于 VP4 蛋白的亚单位疫苗的研究进展进行了综述, 以期对轮状病毒疫苗的发展提供借鉴意义。

**关键词:** 轮状病毒, VP4, 亚单位疫苗, 腹泻

## Research progress in rotavirus VP4 subunit vaccine

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**Abstract:** Rotaviruses are leading causes of worldwide acute diarrhea in children younger than 5 years old, with severe consequence of social and economic burden. Vaccination is the most effective way to control rotavirus infection, however, the licensed rotavirus vaccines are ineffective in some low-income countries of Africa and Asia, where the mortality caused by rotavirus is higher than other areas. In addition, there are also safety concerns such as increased risk of intussusception. Therefore, it is urgent to improve the efficiency and safety of rotavirus vaccine to reduce the morbidity and mortality caused by rotavirus. Till now, many efforts are made to improve the effectiveness of rotavirus vaccines, and the inactive vaccine becomes the main

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trend in the research of rotavirus vaccine. The developments in recombinant rotavirus vaccines, especially in VP4 subunit vaccines are summarized in this review, and it could be helpful to develop effective recombinant rotavirus vaccines in further studies.

**Keywords:** rotavirus, VP4, subunit vaccines, diarrhea

轮状病毒是全球范围内引起 5 岁以下婴幼儿腹泻的主要病原体, 主要通过粪口途径传播, 临床症状包括呕吐、发热、水样便等, 严重时会导致脱水死亡<sup>[1]</sup>。全球范围内, 每年由于轮状病毒感染导致的死亡病例高达 40–60 万<sup>[2]</sup>, 轮状病毒疫苗也被 WHO 列为优先发展的十大疫苗之一。目前已经有两种轮状病毒疫苗 (Rotateq<sup>[3]</sup>和 Rotarix<sup>[4]</sup>) 在全球范围内推广使用, 70 多个国家已经将轮状病毒疫苗纳入免疫规划, 另有多种轮状病毒疫苗在区域范围内使用<sup>[5–6]</sup>, 几种候选疫苗也正在进行临床试验<sup>[7–10]</sup> (表 1)。随着轮状病毒疫苗的推广, 轮状病毒导致的年死亡病例由 40–60 万下降到 20 万左右<sup>[11]</sup>。轮状病毒导致的死亡主要发生在非洲和亚洲等不发达的国家和地区, 但是, 在这些国家和地区已经上市的轮状病毒疫苗的有效性仅为 50% 左右, 显著低于发达国家<sup>[12]</sup>。同时, 目前已经上市的轮状病毒疫苗均为减毒活疫苗, 会增加肠套

叠的风险<sup>[13]</sup>。因此, 更加安全、有效的轮状病毒亚单位疫苗的研究对于进一步降低轮状病毒导致的发病率和死亡率具有重要意义。相比减毒活疫苗, 非复制型疫苗, 特别是亚单位疫苗安全性更高, 是目前轮状病毒疫苗研究的主要方向。

## 1 轮状病毒结构及免疫保护机制

轮状病毒属于呼肠孤病毒科, 轮状病毒属, 无包膜二十面体结构, 直径为 70 nm, 其基因组含 11 条双链 RNA 片段, 分别编码 6 种结构蛋白 (VP1–4、VP6、VP7) 和 6 种非结构蛋白 (NSP1–6)。轮状病毒具有 3 层衣壳结构, 分别是由 VP1、VP2 和 VP3 三种结构蛋白构成的内衣壳, 由 VP6 构成的中间衣壳, 以及由糖蛋白 VP7 和刺突蛋白 VP4 构成的外衣壳<sup>[14]</sup>。

目前, 轮状病毒的免疫保护机制尚不清楚, 一般认为, 固有免疫和获得性免疫在轮状病毒的免疫保护中均发挥着重要作用。轮状

表 1 轮状病毒现有疫苗及候选疫苗的研究

Table 1 Available rotavirus vaccines and rotavirus vaccine candidates

Rotavirus vaccines	Properties	Status	References
Rotarix	Monovalent rotavirus strain G1P[8] RIX4414	Licensed	[3]
Rotateq	Pentavalent reassortant, G1–G4P[8], G6P[7] vaccine of five human-bovine reassortant	Licensed	[4]
LLR	G10P[12]	Licensed in China	[5]
Rotavin-M1	G1P[8]	licensed in Vietnam	[6]
BRV-PV	UK reassortant, G1–G4 and G9	Phase III	[9]
RV3	G3P[6]	Phase II	[7]
116E	G9P[11]	Licensed	[8]
P2-VP8	G1P[8], fusion with a universal T epitope of tetanus toxin	Phase II	[10]
VLPs	Virus-like particles composed of VP2 and VP6, with or without VP4 and VP7	Tested in various animals	[73]
Subunit proteins	VP6, VP4, VP7, NSP4	Tested in various animals	[36–37, 40, 51]

病毒感染后,血清中 IL-6、IL-10 以及 IFN- $\gamma$  水平平均会明显升高,且 IFN- $\gamma$  能够抑制轮状病毒的复制<sup>[15]</sup>。同时,机体会产生针对 VP2、VP4、VP6、VP7 以及 NSP4 等蛋白的抗体。研究表明,针对 VP4 蛋白的抗体可以阻断轮状病毒的吸附与入胞,而针对 VP7 蛋白的抗体则能够阻断轮状病毒的脱壳,从而抑制轮状病毒的复制<sup>[16]</sup>。尽管 VP6 不是中和抗原,但可以刺激机体产生 IgA, IgA 与 pIgR 结合,可以由肠基底侧转运至肠腔,在转运过程中可以与脱去外衣壳的双层病毒颗粒结合,从而抑制轮状病毒的转录和复制<sup>[17]</sup>。自然感染对再次发生轮状病毒感染性腹泻具有一定的保护性,一般情况下,再次感染后无明显症状或症状较轻<sup>[18]</sup>。Chiba 等的研究表明,自然感染的保护性与高滴度的中和抗体有关<sup>[19]</sup>,而后续的研究表明,自然感染的保护性与 IgA 抗体的关系更密切<sup>[20]</sup>。目前已经上市的轮状病毒疫苗的临床结果也表明,轮状病毒疫苗的免疫保护性与 IgA 的水平存在一定的相关性<sup>[21]</sup>。细胞免疫在预防轮状病毒的感染中也发挥着重要作用,但目前仅限于动物模型的研究,与自然感染以及疫苗接种后的保护性的关系尚不清楚。轮状病毒免疫保护机制的研究为发展不同类型的轮状病毒疫苗奠定了理论基础。

## 2 轮状病毒基因工程疫苗研究进展

轮状病毒基因工程疫苗的研究始于 20 世纪 80 年代,包括病毒样颗粒 (VLP) 疫苗<sup>[22]</sup>、重组抗原亚单位疫苗 (VP4<sup>[23]</sup>、VP6<sup>[24]</sup>、VP7<sup>[25]</sup>、NSP4<sup>[26]</sup>)、多肽疫苗<sup>[27]</sup>以及核酸疫苗<sup>[28]</sup>等,其中研究最早的是合成肽疫苗,但其免疫原性较低<sup>[27]</sup>,研究最多的是病毒样颗粒疫苗,而进展最快的则是基于 VP4 蛋白的亚单位疫苗,目前

已经完成了 I 期临床<sup>[10]</sup>。

轮状病毒 VLP 疫苗<sup>[29-35]</sup>、重组 VP6<sup>[36,37]</sup>均在小鼠模型上能够抑制轮状病毒的复制和排毒,具有较高的免疫保护性,但是 VLP 在家兔和无菌猪模型中免疫保护性较低<sup>[31,38]</sup>。另外,研究表明,针对 NSP4 蛋白的抗体可以减轻轮状病毒导致的腹泻<sup>[39]</sup>。VP7 是轮状病毒的主要中和抗原<sup>[40]</sup>,但是,重组表达的 VP7 蛋白不能刺激机体产生高滴度中和抗体<sup>[41]</sup>,这可能与 VP7 为糖蛋白,中和表位构象依赖性较强,而重组表达的 VP7 不能形成正确构象有关<sup>[42]</sup>。

与 VP7 不同,VP4 蛋白没有糖基化修饰,相比 VP7 更容易表达;但是 VP4 作为刺突蛋白,介导了轮状病毒的吸附和入胞过程,其抗体可以阻断轮状病毒的吸附和入胞过程;同时,人源毒株中常见的 P 基因型 (VP4) 仅 P[8]、P[4] 和 P[6] 三种,且 P[8] 占 80% 以上,而常见的 G 基因型 (VP7) 有 G1-G4 以及 G9 五种<sup>[43]</sup>。尽管自然感染以及接种减毒苗后针对 VP4 的中和抗体水平较低<sup>[44]</sup>,但重组表达的 VP4 的免疫原性并不低于 VP7<sup>[45]</sup>,这可能与天然病毒中 VP4 蛋白的含量较低以及 VP5 部分不能充分暴露有关。同时,由于自然感染后针对 VP4 蛋白的抗体水平较低,母源抗体对基于 VP4 蛋白的疫苗的干扰也相对较小。因此,相比 VP7 蛋白,VP4 可能更适合作为轮状病毒基因工程亚单位疫苗的候选抗原。

## 3 重组 VP4 亚单位疫苗的研究

### 3.1 VP4 及其截短蛋白的研究

VP4 蛋白由 776 个氨基酸组成 (人源毒株为 775aa),胰蛋白酶可以将 VP4 切割形成 VP8\* 和 VP5\*,并提高轮状病毒的感染性<sup>[46]</sup>。VP8 蛋白具有血凝素活性,核心结构由 aa65-223 组成,

通过 N 端的柔性区插入 VP5 内部。VP8 蛋白可以与细胞表面的唾液酸受体结合从而介导轮状病毒的吸附<sup>[47]</sup>。轮状病毒吸附后, VP5 蛋白可以与多个细胞受体相互作用并介导轮状病毒的入胞。研究表明, 针对 VP8 蛋白和 VP5 蛋白的抗体均可中和轮状病毒的感染<sup>[48]</sup>。

1987 年, Arias 等通过大肠杆菌将 VP4 蛋白的 N 端 361 个氨基酸 (aa42–387) 与噬菌体聚合酶 MS2 融合表达, 发现 MS2-VP8 能够刺激小鼠产生中和抗体<sup>[49]</sup> (表 2)。1990 年, Mackow 等利用杆状病毒-昆虫细胞表达系统表达了 VP4 蛋白<sup>[51]</sup>, 该蛋白可以被胰酶酶切形成 VP8\* (aa1–246) 及 VP5(1)\* (aa248–474)。小鼠模型的结果表明, VP4、VP8\* 和 VP5 (1)\* 均能刺激小鼠产生中和抗体, 且 VP5 (1)\* 免疫组子代乳鼠腹泻的比例显著低于 VP8\* 免疫组, 说明针对 VP5 蛋白的抗体在体内可以更好地介导免疫保护<sup>[48]</sup>, 这与 Matsui 等之前的研究结果是一致的<sup>[52]</sup>。但是, 多项研究表明, VP5 的免疫原性较低<sup>[50,53]</sup>, 而且重组表达的 VP5 以包涵体形式存在, 不能有效地刺激机体产生中和抗体<sup>[50]</sup>, 因此, 之后的研究主要集中于 VP8 蛋白。

全长的 VP8 蛋白在大肠杆菌中也以包涵体的形式表达, 但其刺激小鼠产生中和抗体的能力与真核表达的 VP8 蛋白无显著差异, 免疫牛和家兔也能产生高滴度的中和抗体<sup>[54]</sup>。将 VP8 蛋白与 GST 融合表达则可以获得可溶性的 VP8 蛋白, 免疫鸡后可以产生高滴度的卵黄抗体<sup>[55]</sup>。2005 年, Mark 等将 VP8 蛋白核心区 (aa65–224) 与 GST 融合表达并解析了 VP8 核心区的结构<sup>[56]</sup>。2012 年, 闻晓波等发现, 在仅融合 6 个组氨酸的情况下, VP8 核心区 ΔVP8 也能够以可溶形式高效表达, 该蛋白在豚鼠模型中可产生高滴度的中和抗

体, 但不能有效地刺激小鼠产生中和抗体<sup>[23]</sup>。2015 年 Xue 等发现, 将 VP8 核心区的 N 端进一步延长至起始于 26 位氨基酸时, 在没有融合蛋白的情况下其可溶性表达量与 VP8 核心区无显著差异, 但其免疫中和活性显著高于 ΔVP8。这可能有两方面的原因, 一方面, VP8 蛋白 N 端柔性区 aa26–65 之间可能存在中和表位, 这与 Jennifer 等 2003 年通过合成肽的方式发现 aa55–66 位可结合免疫血清中的中和抗体相一致; 另一方面, N 端柔性区的存在可能使 VP8 核心区的构象更接近其在天然病毒中的构象<sup>[57]</sup>。

目前, 轮状 VP8 蛋白相关的研究已经较为清楚, 但是, VP4 蛋白相比 VP8\* 和 VP5\* 具有更高的免疫保护性, 一方面, 胰蛋白酶敏感区可以刺激机体产生保护性抗体<sup>[58]</sup>; 另一方面, VP8 存在的条件下, VP5 可能能够被更好地递呈从而产生更高滴度的保护性抗体。同时, VP5 可以刺激机体产生具有交叉中和活性的抗体<sup>[48]</sup>。因此, 包含 VP5 结构域的 VP4 蛋白更适合成为轮状病毒候选基因工程疫苗。

### 3.2 VP4 与外源蛋白的融合表达

尽管截短的 VP4 蛋白可溶性表达量高, 且在弗氏佐剂条件下可以刺激机体产生较高滴度的中和抗体, 但是, 在铝佐剂条件下, 其免疫原性较低, 在小鼠模型中不能有效地刺激机体产生中和抗体<sup>[59]</sup>。为了进一步增强 VP4 的免疫原性, 以介导更强的免疫保护性, 研究人员尝试将 VP4 与能够增强免疫原性的外源蛋白进行融合表达, 如破伤风毒素 T 细胞表位 (P2)、霍乱毒素 B 亚基 (CTB)、大肠杆菌不耐热毒素 B 亚基 (LTB)、布鲁氏杆菌二氧四氢喋呤合成酶 (BLS) 以及颗粒性蛋白鼠多瘤病毒 VP1 和截短的诸如病毒衣壳蛋白 (可形成 P 颗粒) 等 (表 3)。

表 2 VP4 及其截短蛋白在动物模型中免疫原性和免疫保护性的研究

Table 2 The immunogenicity and protective efficacy of VP4 and truncated VP4 in animal models						
Antigens	Strains	VP4 peptides	Adjuvants	Immunization routes	Animal models	References
MS2-VP8*	SA11	42-387	Freund's	Subcutaneously	Mice	[49]
MS2-VP5*	SA11	389-776	Freund's	Subcutaneously	Mice	[50]
VP4	RRV	1-776	Freund's	Intraperitoneal	CD-1 mouse	[48,71]
VP5 (1)*	RRV	247-474	Freund's	Intraperitoneal	Maternal antibody	[48]
VP8*	RRV	1-246	Freund's	Intraperitoneal	Model	[48]
rVP8*	C486	1-231	Freund's	Subcutaneously	Rabbit	[54]
rVP8*	C486	1-231	VSA	Subcutaneously	Cattle	[54]
rVP8*-GST	Wa	1-231	Freund's	Intramuscular	Chicken	[55]
ΔVP8	Wa, DS-1, 1076	65-223	Aluminum phosphate	Intramuscular	Guinea pig	[23]
VP8	LLR	1-231	Freund's	Subcutaneously	BALB/c mouse	[54-55, 57]
VP8-1	LLR	26-231	Freund's	Subcutaneously	Maternal antibody	[57]
ΔVP8	LLR	65-231	Freund's	Subcutaneously	Model	[57]

表 3 VP4 融合表达蛋白在动物模型中免疫原性和免疫保护性的研究

Table 3 The immunogenicity and protective efficacy of VP4 fusion proteins in animal models					
Fusion proteins	VP4 peptides	Strains	Location of fusion protein	Neutralizing antibody	References
BLS	aa62-224	C486	N terminal	BLS-VP8d: 1:794 VP8d: 1:126	BLS-VP8 group significantly decreased Diarrhea [61]
P Particle	aa65-222	EDIM	Insert in the middle	No difference between PP-mVP8 and mVP8	Protection in virus shedding, no difference between PP-mVP8 and mVP8 [62]
P2	aa65-223	Wa	Insert in the middle	Intranasal: PP-VP8>VP8 Subcutaneously: PP-VP8>VP8	No protection effect was observed for EDIM infection [63-64, 71]
VP1	aa65-224	DS-1	Insert in the middle	P2-P[8]ΔVP8: 1:10 240 P[8]ΔVP8: 1:7 241 P2-P[6]ΔVP8: 1:57 926 P[6]ΔVP8: 1:57 926	—
CTB	aa26-231	LLR	N / C terminal	CTB-VP8-1: 1:5 026 VP8-1-CTB: 1:3 411 VP8-1: 1:596	Protection in diarrhea, CTB-VP8 was better than VP8-CTB [72] [59]

在这些融合抗原中, CTB 和 BLS 能够显著提高 VP4 蛋白的免疫原性, 而诺如病毒 P 蛋白和破伤风毒素 T 细胞表位 P2 以及 LTB 对 VP4 免疫原性的影响相对较小<sup>[60]</sup>。VP8 与 CTB 融合表达, 免疫三针后血清中和抗体滴度相比单纯的 VP8 蛋白可以提高 4–8 倍。相比 N 端融合, VP8 融合于 CTB 蛋白 C 端的免疫血清中和滴度更高, CTB-VP8 蛋白免疫组子代乳鼠的腹泻程度也显著低于 VP8-CTB 和单独的 VP8<sup>[59]</sup>。VP8 与 BLS 融合表达, 其免疫血清中和滴度可提高 1 个数量级以上, 且融合蛋白免疫对子代小鼠的被动保护效果显著优于单独的 VP8 免疫组及混合免疫组<sup>[61]</sup>。PP-VP8 仅在滴鼻免疫条件下能够显著提高 VP8 的免疫原性, 但经皮下途径进行免疫时, PP-VP8 与 VP8 的免疫原性无显著差异<sup>[62]</sup>。

P2-VP8 仅在免疫两针后具有更高的血清抗体滴度, 免疫三针后则与 VP8 无显著差异<sup>[63-64]</sup>。尽管如此, P2-VP8 的研究进展最快, 是目前唯一完成 I 期临床的轮状病毒基因工程疫苗。I 期临床的结果显示, 三针免疫后, 所有志愿者的血清 IgA 水平均出现 4 倍及以上升高。但是, 中和抗体的应答率仅为 50%–66.7%, 此外, I 期临床实验受试人群为 18–45 周岁的成年人, 在免疫系统发育尚不完全的婴幼儿中, P2-VP8 的免疫原性和免疫保护性还需要进一步的研究<sup>[10]</sup>。

### 3.3 其他

近年来, 也有研究团队通过植物表达轮状病毒 VP8 蛋白。2004 年, Filgueira 等以烟草花叶病毒作为载体在烟草中表达了牛轮状病毒 VP8 蛋白, 并且在小鼠模型中体现出一定的免疫保护性<sup>[65]</sup>。2011 年, Lantz 等通过转基因的方式在烟草的叶绿体中表达了牛轮状病毒的 VP8

蛋白, 该蛋白免疫小鼠可产生高滴度的中和抗体, 并且对子代乳鼠的腹泻具有 80% 以上的保护率<sup>[66]</sup>。2015 年 Federico 等发现, 将 VP8 与 BLS 融合表达免疫母鸡能够产生高滴度的卵黄抗体, 但是冻干后其免疫原性有一定程度的降低<sup>[67]</sup>。尽管植物的生产成本较低, 但是目前植物表达系统尚不完善, 病毒载体和转基因的方式均存在一定的局限性, 即使目标蛋白能够成功表达, 提取和纯化的难度也很大, 因此, 通过转基因植物生产疫苗还存在一定的挑战。

此外, 通过可食用的乳酸菌也可以表达轮状病毒的 VP4 蛋白及其 VP8 结构域, 通过口服方式进行免疫后, 这些改造后的乳酸菌可以刺激小鼠产生一定的中和抗体, 但是滴度较低<sup>[60,68-69]</sup>。因此, 尽管乳酸菌提供了一种安全、简便的方式, 为发展新型的黏膜免疫疫苗提供了新的选择, 但是, 由于其免疫原性较低, 是否能够成为候选疫苗还有待进一步研究。

## 4 结语

轮状病毒感染是一个全球性的公共卫生问题, 轮状病毒疫苗已经上市并纳入多个国家的免疫规划。随着轮状病毒疫苗的接种, 轮状病毒导致的发病率和死亡率显著下降, 但是, 在非洲和亚洲等轮状病毒导致的死亡率较高的发展中国家, 轮状病毒疫苗的保护率仍有待进一步提高。高滴度的母源抗体是导致轮状病毒减毒疫苗有效性低的主要因素之一。尽管 VP4 介导了轮状病毒的吸附和入胞过程, 自然感染中产生的针对 VP4 蛋白的抗体远低于 VP7。因此, 基于 VP4 蛋白的轮状病毒疫苗有希望能够打破母源抗体的干扰, 在母源抗体较高的发展中国家

家和地区产生更好的免疫保护效果。但是, VP4 亚单位疫苗的研究目前仍存在以下三方面的问题: 1) 不同于天然病毒和 VLP 疫苗, VP4 亚单位疫苗的免疫原性较弱; 2) 不同毒株受体识别的差异可能会导致轮状病毒疫苗对不同毒株保护性的差异; 3) 缺少有效的动物模型。轮状病毒基因工程疫苗在不同动物模型中的免疫保护性差异较大, 且缺少能够有效反映轮状病毒疫苗有效性的血清学指标, 限制了轮状病毒疫苗研究。此外, 新的流行毒株的出现也为轮状病毒疫苗的研究带来了挑战。因此, 进一步提高 VP4 蛋白的免疫原性, 加快轮状病毒动物模型的研究才能使 VP4 亚单位疫苗成为可能。

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